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RESEARCH REPORT

The genetic diversity and differentiation of shrimp *Fenneropenaeus chinensis* in the Yellow Sea revealed by polymorphism in control region of mitochondrial DNA

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Abstract

Chinese white shrimp *Fenneropenaeus chinensis* is a commercially important species in northern China and Korea. In the present study, the genetic diversity of five populations collected from Qingdao (QD), Rizhao (RZ) of China, and Narodo Island (KN), Taean (KT), Yeongguang (KY) of Korea in the Yellow Sea was investigated using the mitochondrial control region (CR). The length of the amplified partial mitochondrial control region (mtCR) ranged from 600 to 622 bp, and the sequence variations were distributed among 13 polymorphic sites. The pattern of nucleotide substitution was biased in favour of transitions over transversions in variable sites, including 12 transitions (si, 4 A↔G and 8 T↔C changes) and only one was transversion (sv, 1 T↔G changes). Altogether, 24 unique haplotypes were identified from five populations in Yellow Sea. The overall haplotype diversity and nucleotide diversity were 0.368 - 0.421 and 0.052 - 0.079, respectively, and the lowest genetic diversity was found in QD population. There was no differentiation between the two Chinese populations (F_{ST} = 0.039). Within the Korean populations, there was a slight differentiation (F_{ST} = 0.075, $p < 0.05$) between KN and KT. The relative bigger differentiation was shown between RZ and KN population (F_{ST} = 0.170, $p < 0.05$). The relative further genetic distance was shown between RZ and KN population as well as between QD and KN population, while the relative closer genetic distance was shown between KT and KY, and between KT and RZ population. The low variability in the mitochondrial control region among *F. chinensis* in the Yellow Sea indicated the low genetic diversity in comparison to other shrimp species. The results suggested a slight population differentiation among *F. chinensis* populations. Such information will assist in sustainable use, management, and conservation of the species.

Key Words: *Fenneropenaeus chinensis*; control region; genetic diversity; population genetics; polymorphism

Introduction

The Chinese shrimp *Fenneropenaeus chinensis*, mostly distributed in the Yellow Sea, Bohai Sea in China and the west coast of the Korean Peninsula (Liu, 1959, 1990). It has been playing an economically important role in the fishing and farming industries in northern China (Ye, 1984, 1994; Xin, 1999). The Chinese shrimp aquaculture industry suffered a severe mortality problem caused by shrimp white spot syndrome virus in the middle of 1990s, and fishing production also decreased significantly (Liu, 2003; Guo, 2006). Shrimp larvae have been released annually into the Yellow Sea over the last two decades to replenish the decreasing shrimp stocks in China. However, such large scale restocking of hatchery raised larvae and the escape of farmed individuals can significantly affect the genetic structure of shrimp population in Yellow Sea. *F. chinensis* wild stocks are further threatened by overfishing, viral epizootics, and habitat contamination.

A better understanding of population structure is important to the effective fisheries management and conservation of genetic resources in exploited marine organisms (Rolda'n *et al.,* 2000). Recent researches on the genetic variation within *F. chinensis* have utilized new analytical and technical tools that provide high-resolution genetic information.

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Table 1 Shrimp *Fenneropenaeus chinensis* populations sampled for present study

Sampling locality	Abbrev.	Sample for sequencing	Average wet weight (g)
Narodo Island, Korea	KN	23	94.37
Yeongguang, Korea	KY	25	60.25
Taean, Korea	KT	20	70.75
Qingdao, China	QD	12	26.13
Rizhao, China	RZ	20	46.45

Two geographic populations of *F. chinensis* had previously been defined, one along the coast of northern China and the other on the west coast of the Korean Peninsula (Kim, 1973; Deng *et al.,* 1983, 1990; Zhuang *et al*., 2001). Recently, a new population of *F. chinensis* was found near the Jeju Island in southern Korea, called the southern coast population of the Korean peninsula (Liu *et al.,* 2004, 2006). Its spawning location and migration routes are different from the others.

Most previous researches revealed the very low genetic diversity of *F. chinensis* in Yellow Sea and Bohai Sea (Liu *et al.,* 2000a b; Qiu *et al*., 2001; Ma *et al*., 2004; Meng *et al*., 2004; Cui *et al*., 2007) using various approaches such as Radom Amplified Polymorphic DNA (RAPD) and microsatellite DNA. The sequence and structure of mitochondrial genomes (mtgenomes) are gaining increasing popularity in higher-level phylogenetic analysis because of their ability to provide better resolution for relationships than single or multi-gene analysis and the relative ease of sequencing of the entire genome, which can provide information on phylogenetic relationships and on the genetic structure of populations and patterns of gene flow (Cameron *et al*., 2004). This information may derive from gene order, the sequences of individual genes, restriction fragment length polymorphism (RFLP) analysis of mtDNA, or the sequences of complete genomes. Although there are various reports on the genetic diversity of *F. chinensis* and population differentiation by using different DNA markers, their resolutions are not sufficient to discriminate the less genetically differentiated populations. The mitochondrial 16S rRNA and cytochrome oxidase I (COI) genes were often used in elucidating population structure of penaeid shrimps over a broad geographic range (Klinbunga *et al.*, 2001; Tsoi *et al.*, 2007), but they were found less useful in identifying genetic difference over smaller geographic scales (Tsoi *et al*., 2007), as in the case of *F. chinensis*.

In the present study, the population structure of shrimp (*F. chinensis*) in the Yellow Sea was inferred using highly variable control region of mitochondrial DNA to provide more information on the genetic diversity of *F. chinensis* for the sustainable utilization of its wild stocks, the management of fisheries and the health of ecosystem in the Yellow Sea in terms of genetic diversity in exploited stocks.

Materials and Methods

Sample collection and DNA extraction

Shrimps *F. chinensis* were collected from the following five localities (Table 1, Fig. 1): Taean (abbreviated to KT, Chungnam-do,), Narodo Island (KN, Jeollanam-do), and Yeongguang (KY, Jeollanam-do) from the Korean Peninsula in the Yellow Sea; and Qingdao (QD, Shandong), Rizhao (RZ, Shandong) from the coast of northern China in the Yellow Sea. The specimens were kept at -20 °C until analysis. Total genomic DNA was isolated from approximately 20 - 50 mg of pleopod tissue that was minced and digested at 55 °C for 3 h in 500 μl of extraction buffer (10mM Tris/HCl at pH 8.0, 50 mM

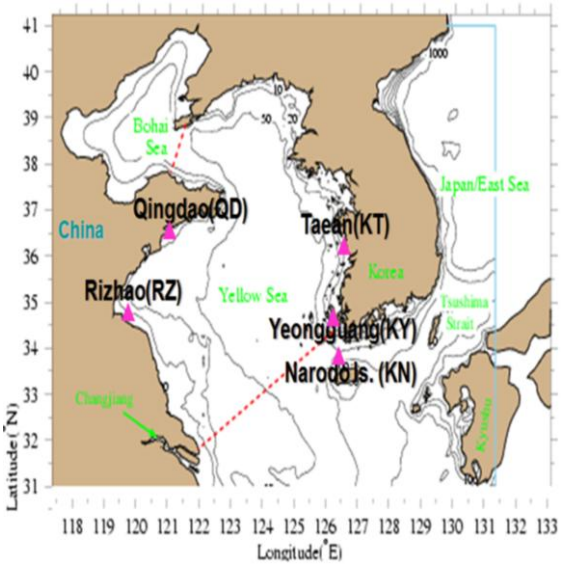

Fig. 1 Purple triangles indicate sampling locations of five shrimp populations: Taean (abbreviated to KT, Chungnam-do), Narodo Island (KN, Jeollanam-do), and Yeongguang (KY, Jeollanam-do) from the Korean Peninsula in the Yellow Sea; and Qingdao (QD, Shandong), Rizhao (RZ, Shandong) from the coast of northern China in the Yellow Sea (referenced from Wang *et al.,* 2006).

EDTA, 1 % sodium dodecyl sulfate and 100 μg ml^{-1} proteinase K). The mixture was then extracted twice with phenol, phenol/chloroform (1:1) and once with chloroform/isoamyl alcohol (24:1), followed by ethanol precipitation. The samples were washed with 70 % ethanol, air-dried, and re-dissolved in distilled water. The detailed sampling location, sampling size and other information were shown in Table 1 and Figure 1.

Sequencing of partial control region

A total of 100 Shrimps were employed for amplification of the 5' segment of mitochondrial CR using the primers 12S (5'-AAG AAC CAG CTA GGA TAA AAC TTT-3') and PCR-1R (5'-GAT CAA AGA ACA TTC TTT AAC TAC-3') (Chu *et al*., 2003). The reaction mixture (20 ml) contained ~100 ng of DNA template, 0.2 mM of each primer, 1×thermophilic DNA polymerase buffer, and 2.5 U Taq DNA polymerase (Qiagen). The thermal cycling profile for amplification was one cycle of 3 min at 94 °C, 35 cycles of 40 s at 94 °C, 40 s at 48 °C, and 30 s at 72 °C, and a final extension at 72 °C for 3 min. Single bands of the predicted size were obtained from PCR. Prior to sequencing, PCR products were purified using either the QIAquick PCR purification kit or gel purification kit according to the manufacturer's instructions (Qiagen). Sequences of purified PCR products were obtained from both directions using the same primers for PCR.

Sequence assembly and annotation

Sequences from both strands in each specimen were aligned with CLUSTAL X1.81 (Thompson *et al.,* 1997) and individual consensus sequences were retrieved with both alignment and manual check. Contig sequences were checked for ambiguous base calls and only non-ambiguous regions were used for annotation. The partial control region sequences of the *F. chinensis* were compared with those submitted to GenBank, and BLAST database searches were performed to make sure correct target sequences amplified.

Software computation

Molecular diversity indices such as number of haplotypes, polymorphic sites, transitions, transversions and indels were obtained using Arlequin (Ver. 2.000, Schneider *et al*., 2000). Haplotype diversity (H), nucleotide diversity (π), and their corresponding variances were calculated following Nei (1987) as implemented in Arlequin. The amounts of genetic variability partitioned within and among populations were accessed by an analysis of molecular variance (AMOVA; Excoffier *et al*., 1992). Significance of pairwise population comparison was tested by 20,000 permutations. Organization of the AMOVA tests was in a hierarchical manner and 1,000 permutation procedures were used to construct null distributions and to test the significance of variance components (Guo and Thompson, 1992). AMOVA and bootstrap analysis with 5,000 replicates were performed in Arlequin. The inclusive Tamura-Nei (TrN) (Tamura and Nei, 1993) model was used to calculate the genetic pairwise distances between haplotypes. The isolation-by-distance effects on population genetic structure were estimated by pairwise F_{ST} statistics (Wright, 1951, 1965). The significance (5 % level) of the F_{ST} was tested by 1,000 permutations for each pairwise comparison.

Phylogenetic trees of the haplotypes were constructed using MEGA 4.0 (Tamura *et al*., 2007). The neighbour-joining (NJ) algorithm (Saitou and Nei, 1987) was implemented to construct a phylogenetic tree from the maximum likelihood (ML) distances estimated under the selected models. Relationships between haplotypes were also determined with the Kimura two-parameter distance model by using the neighbour-joining method in MEGA 4.0. Bootstrap analysis with 1,000 replicates was used to evaluate reliability of phylogenetic relationships (Felsenstein, 1985).

Results

Sequence variation

PCR products from the mitochondrial control region of 100 individuals were obtained with the primers 12S and PCR-1R, which ranged from 600 to 622 bp (including primers). The nucleotide composition of this fragment consisted of 9.55% cytosine, 45.06 % thymine, 37.04 % adenine, and 8.35 % guanine. Of the partial mitochondrial control region (mtCR) sequences, the sequence variations were distributed among 13 polymorphic sites (Table 2).

The pattern of nucleotide substitution was biased in favour of transitions over transversions in variable sites, including 12 transitions (si, 4 A↔G and 8 T↔C changes) and only one was transversion (sv, 1 T↔G changes) (Table 2). Altogether, 24 unique haplotypes were identified from five populations in Yellow Sea (Table 2). Haplotype KY12 was the most common and was observed in all samples, and its frequency was 24 % in the total samples. Another two haplotypes (KN02, QD12) occurred frequently in five populations, and KN02 showed higher frequency in Korean Population than that in Chinese population. Haplotype RZ08, RZ11 and RZ16 were shared by the QD and RZ samples from the Chinese Coast. The distribution of ten mtCR haplotypes was restricted to only one population. And they were sample-specific haplotypes. None of the haplotypes was shared only among the populations from the region of Korean coast (KN, KY and KT). The population pairs and the numbers of haplotypes shared between the populations from Chinese and Korean coast were as follows: KN and QD populations (five); KN and RZ populations (five); KT and QD populations (six); KT and RZ populations (Four); KY and RZ populations (Four); KY and QD populations (Six). Haplotype frequencies of CR fragment and their distributions in the five samples are shown in Table 2.

Genetic diversity and population structure

The number of haplotypes, haplotype diversity (H), and nucleotide diversity (π) for each population were listed in Table 3. The genetic variation level was low, whether measured as haplotype diversity (0.368 - 0.421) or nucleotide diversity (0.052 - 0.079). The lowest genetic diversity was found in QD population from Qingdao. And KN population from

Table 2 Variable nucleotide positions defining the mtDNA control region haplotype from each of five shrimp population sampled (sequence identity to reference sequence in top row KN02), haplotype frequencies in 5 shrimp populations and total number of individuals of each haplotype (N).

Haplotype	Variable sites and positions													Populations					
	7 1	7 7	3 1 5	3 3 4	3 6 4	3 8 6	4 0 0	4 1 3	4 2 0	4 2 1	4 2 2	4 4 2	5 6 2	KN	KY	KT	QD	RZ	N
KN 01	A	T	T	G	T	T	T	T	T	A	T	A	C	**1**	**2**	**1**	**2**	0	6
KN02	A	G	T	G	C	T	T	T	C	A	T	A	T	**5**	**3**	**3**	**2**	**3**	16
KN13	A	G	C	G	C	T	T	T	C	A	T	A	T	**1**	0	0	0	0	1
KN17	A	T	T	A	C	T	T	T	T	A	T	G	T	**1**	0	0	0	0	1
KN19	A	G	T	G	C	T	C	T	T	A	T	A	T	**1**	0	0	0	**1**	2
KY02	A	T	C	G	T	T	T	T	T	A	T	A	C	0	**1**	0	0	0	1
KY06	A	T	T	G	T	T	T	T	T	A	T	A	T	0	**1**	0	0	0	1
KY12	G	G	C	G	C	T	T	T	C	A	T	A	T	**9**	**8**	**4**	**2**	**1**	24
KY20	A	G	T	G	C	T	T	T	T	A	T	A	T	**1**	**1**	0	0	0	2
KY21	G	G	C	G	C	T	T	T	C	A	C	A	T	0	**1**	0	0	0	1
KT12	A	T	T	G	C	T	T	T	T	A	T	A	T	**1**	0	**1**	0	0	2
KT13	A	T	C	G	C	T	T	T	T	A	C	A	T	0	0	**1**	0	0	1
KT19	A	G	T	G	C	C	T	T	T	G	T	A	T	**1**	0	**1**	0	0	2
KT22	A	T	T	A	C	T	T	T	T	G	T	A	T	0	**1**	**1**	0	4	6
KT23	A	G	T	G	C	T	T	T	C	A	C	A	T	0	0	**1**	0	0	1
QD12	A	T	T	G	T	T	T	T	T	A	C	A	C	**1**	**3**	**2**	**1**	**4**	11
QD25	G	G	C	G	C	T	T	T	C	A	T	G	T	**1**	0	0	**2**	**1**	4
QD30	A	T	T	G	C	T	T	C	T	A	T	A	T	0	**1**	**2**	**1**	0	4
QD37	A	T	T	G	C	C	C	T	T	A	T	A	T	0	**3**	**3**	**1**	0	7
RZ01	A	T	C	G	C	T	T	T	T	A	T	A	T	0	0	0	0	**1**	1
RZ05	A	G	T	G	C	T	T	T	C	A	T	G	T	0	0	0	**1**	**1**	2
RZ08	A	T	T	G	T	T	T	T	T	A	C	G	C	0	0	0	0	**1**	1
RZ11	A	T	T	G	C	T	T	C	T	A	C	A	T	0	0	0	0	**2**	2
RZ16	A	T	T	A	C	T	T	T	T	G	T	G	T	0	0	0	0	**1**	1
Total	12 Transition, 1 Transversion													23	25	20	12	20	

Narodo Island, Korea also showed lower genetic variation level than RZ (Rizhao) population in China, and other KT and KY populations from the Korean side.

When the samples were pooled into different regional groupings, AMOVA analysis showed that more than 93 % of the total molecular variance was distributed within populations (Table 4). The F_{ST} values for Korean (KT, KN, KY) and Chinese groups (RZ, QD) was 0.044 and the F_{ST} value for north (QD, KT) and south groupings (RZ, KN, KY) was 0.021 therefore indicated no significant differentiation. However, the F_{ST} value obtained when comparing the southern Korean population (KN) with the rest (QD, KT, RZ, KY) was 0.069, and the F_{ST} value obtained when comparing KN, QD with the rest (KT, RZ, KY) was 0.058 (Table 4), indicating a slight population differentiation throughout the range of *F. chinensis* populations in Yellow Sea.

Genetic differentiation between populations

Genetic differentiation among shrimp populations was assessed using F_{ST} pairwise comparisons. In the 10 possible comparisons, four of the pairwise F_{ST} estimates were negative (Table 5), indicating that the variation within samples was greater than variation between samples. There was no differentiation between the two Chinese populations (F_{ST} = 0.039). Within the Korean populations, there was a slight differentiation (F_{ST} = 0.075) between KN and KT (p < 0.05). And the KN and RZ population showed a severe differentiation (p < 0.05) (F_{ST} = 0.170). The relative further genetic distance was shown between RZ and KN population as well as between QD and KN population, while the relative closer genetic distance was shown between KT and KY, and between KT and RZ population (Table 6, Fig. 2). The relationship among all the 24 haplotypes was shown in Figure 3. All the 24 haplotypes were clustered with two separate groups. Three haplotypes (KY12, KN02 and QD12) were found in all samples, while ten mtCR haplotypes were restricted to one population. Eleven mtCR haplotypes were shared in any two of the shrimp populations.

Table 3 Summary of molecular diversity for *F. chinensis*, h, haplotype diversity, π, nucleotide diversity

Population	No. of haplotype	*h*	Π
KN	5	0.372	0.052
KY	5	0.397	0.073
KT	5	0.421	0.078
QD	4	0.368	0.052
RZ	5	0.416	0.078

Discussion

In general, the genetic diversity of *F. chinensis* in the Yellow Sea appears to have diminished in the past years. The heterozygosity has decreased from

Table 4 The Amova analysis of shrimp populations in different groups.

Source of variation	Sum of squares	Variance components	Percentage variation	P values	Fst
Group (KY, KT, KN) and group (QD, RZ)					
Between groups	4.118	0.02726	1.41	0.00000	0.044
Between populations within groups	8.999	0.05753	2.98	0.00000	
Within populations	175.392	1.84624	95.61	0.00933	
Total	188.510	1.93103			
Group (QD, KT) and group (RZ, KN, KY)					
Between groups	0.786	-0.07335	-3.89	0.00000	0.021
Between populations within groups	12.332	0.11294	5.99	0.00000	
Within populations	175.392	1.84624	97.90	0.00387	
Total	188.510	1.88583			
Group (QD, KT, RZ, KY) and group (KN)					
Between groups	6.394	0.11517	5.81	0.00000	0.069
Between populations within groups	6.723	0.02092	1.06	0.00000	
Within populations	175.392	1.84624	93.13	0.01153	
Total	188.510	1.98233			
Group (KT, RZ, KY) and group (QD, KN)					
Between groups	6.329	0.08972	4.70	0.00000	0.058
Between populations within groups	6.702	0.02230	1.17	0.00000	
Within populations	170.669	1.79652	94.13	0.01045	
Total	183.700	1.90854			

Table 5 Pairwise difference among shrimp populations (The significance (5 % level) of the F_{ST} was tested by 1,000 permutations for each pairwise comparison)

Population	KN	KY	KT	QD	RZ
KN	0.00000				
KY	0.03065	0.00000			
KT	0.07510*	-0.01863	0.00000		
QD	-0.00094	-0.03762	-0.01306	0.00000	
RZ	0.17016*	0.04899	0.00763	0.03887	0.00000

1995 to 1998 based on allozyme analysis, and the percentage of polymorphism loci and heterozygosity has also decreased from 1997 to 2001 based on RAPD analysis. An investigation of the genetic variation within this species will provide useful information that can be used to manage shrimp stocks and protect genetic variation in this species.

The control region in mtDNA is a non-coding DNA area, which is the most polymorphic region of mtDNA genome. Of the partial mitochondrial control region (mtCR) sequences identified in the present study, sequence variations were distributed among 13 polymorphic sites across five populations. The pattern of nucleotide substitution in *F. chinensis* was biased in favour of transitions over transversions in variable sites, consistent with the bias in favour of transitions characteristics of the control region in other animal species (Brown *et al*., 1982; Kocher *et al.,* 1989; Ram´ırez-Mac´ıas *et al*., 2007). Similar to the results of others (Shi, 1999; Song, 1999; Wang, 2001; Liu *et al*., 2004; Cui *et al*., 2007), the present results indicated that the genetic diversity of *F. chinensis* was low, and maybe the lowest among *Penaeus* species studied so far (Hualkasin *et al*., 2003; McMillen-Jackson and Bert, 2003, 2004; Tzeng *et al.,* 2004; Table 3). The low genetic diversity found in the population KN from Narodo Island near the Jeju Island in Korea might indicate southern coast population of the Korean peninsula to be somewhat different from populations in other areas (Liu *et al*., 2004). As different methods were used in the prior studies, it was difficult to draw a conclusion whether there was a decrease or increase in genetic diversity compared with the current study. Even though the same mtCR was used in the present study and in an earlier one (Cui *et al*., 2007), the data were not comparable as the mtCR information was derived differently from restriction fragment length polymorphism (RFLP) analysis and the sequencing of partial control region. Though the estimated mutation rate was different when using different mtDNA markers, the level of genetic variation in Chinese shrimp was still low. The lower diversity of Chinese Qingdao population in comparison with the RZ, KY and KT populations, suggested that QD population had undergone a recent population bottleneck or founder event and might has lost some specific alleles. The reduced genetic diversity of *F. chinensis* in China appeared to be due to the use of a small pool of broodstock during decades of extensive prawn farming and release of the excess postlarvae on a large-scale for stock enhancement (Zhuang *et al.,* 2001). Integration of genetic diversity data from the present study and previous research indicated that the *F. chinensis* populations bore low genetic diversity in contrast to other Penaeus species, likely due to the reduction of effective population size arising from habitat instability during sea-level variations. Because the extent of genetic variation is closely related to the evolution of organisms, food supply and the ability to withstand adverse environment, the low genetic diversity not only challenges the future existence of *F. chinensis*, but is also one of the reasons why *F. chinensis* has low resistance against disease and adverse environments.

Table 6 Genetic distances (Tamura-Nei method) between five populations of *F. chinensis* from control region sequences

Population	QD	RZ	KT	KY	KN
QD	--				
RZ	0.007	--			
KT	0.006	0.005	--		
KY	0.007	0.007	0.005	--	
KN	0.011	0.015	0.008	0.009	--

In the present study, AMOVA analysis showed most of the total molecular variance was distributed within populations at different hierarchical levels. All the conventional population F_{ST} statistics showed significant difference between the population KN and the other four populations as a north group. Within the Korean populations, there was a slight differentiation between KN and KT. And the KN and RZ population showed a severe differentiation. There was no differentiation between the two Chinese populations from the yellow sea. These results were consistent with the opinions that there were two geographic populations of *F. chinensis* in the Yellow Sea and the Bohai Sea from recapture data (Deng *et al*., 1990), and the variation among Korea and Chinese populations was larger than the variation within populations, indicating the possible differentiation among the two populations (Shi *et al.*, 1999). In addition, Meng *et al.* (2004) found over three quarters of variation occurred within samples from the Yellow Sea and the Bohai Sea, and this differentiation had taken place to some extent among *F. chinensis*. The high F_{ST} statistics between the population KN and the other four populations as a north group indicated the differentiation between them. Although the sampling area and the sampling season were not so strict, (the sampling date for KN, KY, KT was April, May, June in 2008, respectively, and the RZ and QD were sampled in August 2008), the results from the present study favoured the definition of a new *F. chinensis* population near southern Korea suggested by a previous study with a different spawning location and migration route from the others (Liu *et al.,* 2004).

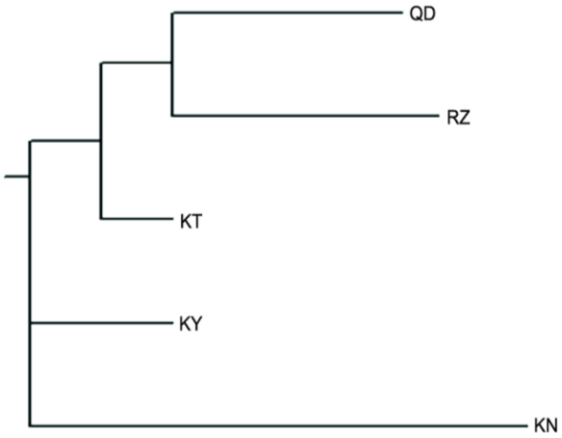

Fig. 2 Neighbour-joining tree of five populations for shrimp *F. chinensis*.

Cui *et al.* (2007) pointed out that there was little genetic differentiation among *F. chinensis* populations because of extensive gene flow. In the present study, three haplotypes (KY12, KN02 and QD12) were found in all samples, indicating a common source of origin for these populations and eleven mtCR haplotypes were shared in any two of the shrimp populations suggesting that gene flow was likely to have occurred between samples as a result of the long migrations and wide

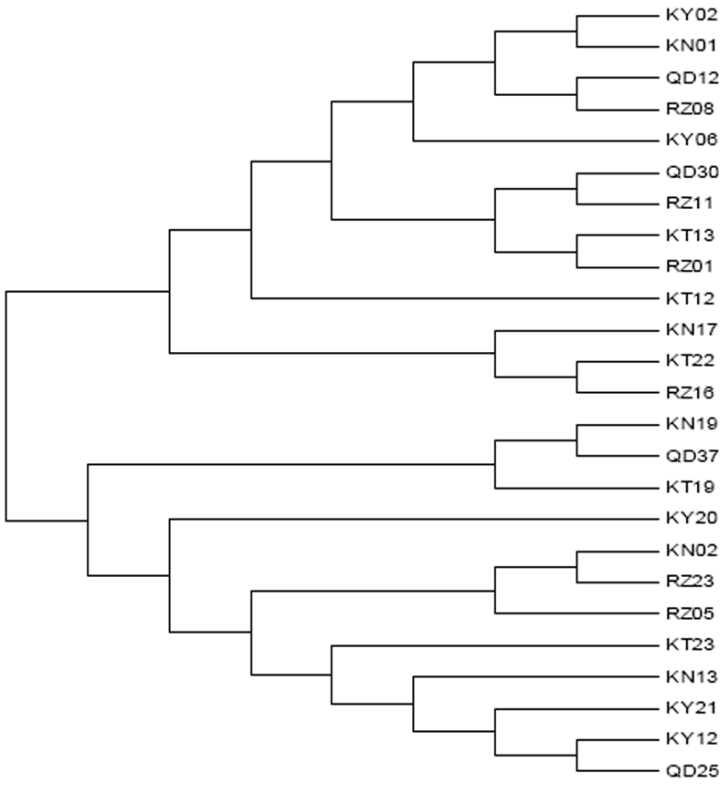

Fig. 3 Neighbour-joining tree of mtDNA control region haplotypes of shrimp *F. chinensis*.

dispersal of these shrimps. Instances of uniform marine populations are regarded to be due either to non-equilibrium populations or to a truly high degree of larval dispersal (Palumbi, 2003). However, ten mtCR haplotypes were restricted to one population suggesting there were some barriers between populations, and this was consistant with the previous study by Meng *et al.* (2009) which suggested that the persistent reproductive isolation had likely contributed to the genetic differentiation among geographic populations of *F. chinensis* in the Yellow and Bohai Sea. With regard to the origin/formation and genetic background of KN were still unknown, different opinions about the genetic differentiation of *F. chinensis* nevertheless remain. In any case, it is undeniable that low genetic divergence will lead to germplasm depression characterized by slow growth rate, reduced productivity and disease susceptibility (Zhang *et al.* 2002).

The mtDNA variations of diversity in this species are the legacy of historical events. The current study favoured the previous result from Liu *et al.* (2004), in which a new population of *F. chinensis* was found around the Jeju Island near southern Korea. Given the differences in spawning, mating, migrating time and over-wintering places, *F. chinensis* may have three geographic populations: the Yellow and Bohai Sea (YB) coast population, the western Korean peninsula (KW) coast population, and the southern coast of Korean Peninsula (KS). *F. chinensis* is also found in small quantities near the Shengsi and Zhoushan archipelago in the north part of the East China Sea and the mouth of the Pear River in the South China Sea (Liu and zhong, 1988). However the amount of *F. chinensis* fished in the East China Sea and the South China Sea was very low and usually mixed with *Fenneropenaeus penicillatus* and other *Penaeus* species (Liu *et al.,* 1959, 1988). Unlike those individuals in the Bohai Sea and the Yellow Sea, which usually migrate twice a year for reproduction and over-wintering during their life cycles , individuals of *F. chinensis* in the South China Sea only move within a small area and show no long distance migration (Liu and Zhong,1988). This unique behaviour of *F. chinensis* in the Bohai Sea and the Yellow Sea was presumed to have resulted from gradual adaptation during the process of evolution. Given the highly migratory nature of *F. chinensis* in Bohai Sea and Yellow Sea, and the lowly migratory nature of *F. chinensis* in East China Sea and South China Sea, some tagging studies centred on the spawning location and migration routes for *F. chinensis* from these areas are crucial to fill important gaps on *F. chinensis* biology, and particularly migration patterns.

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